IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Takeya ABE et al.

Application No.: 09/936,514

Group Art Unit : 1652

Filed : 09/14/2001

Examiner : Fronda, Christian L

Title : PROCESS FOR PURIFYING AMIDE COMPOUND

DECLARATION UNDER 37 C.F.R 1.132

Honorable Commissioner of Patents and Trademarks

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Washington, D.C. 20231

Sir:

I, Kiyoshi ITO is one of inventors of the present invention and familiar with the subject matter of above identified application and the cited references WO 99/55719 and Biol. Chem. 1967.Jan. 25: 242(2); 173-81 and declare and state:

(1) That I have been engaged in research concerning novel Nitrile Hydratase development from *Pseudonocardia thermophila* JCM3095 and its application activity for novel Acrylamide production process.

(2) That I have been employed by Mitsui Chemical Co., since April 1989.

(3) That I graduated from the Faculty of Agriculture ,the University of Tokyo in a master course in March of 1989.

(4) That the following experiments were carried out by myself or under my direct supervision and control in order to show the differences between the subject matter of the reference and the subject matter of the application:

Experiments

in order to establish unexpected results when the process for purifying an amide compound is carried out under pH range according to claim 1, following experiments were carried out.

In the experiments, HPLC analysis of a reaction solution is carried out by using ultron PS 80HG(50×8mmϕ) as a column and 10mM phosphoric acid solution as a developer, and acrylamide is detected by optical absorbance at 220 nm. In order to confirm the effect of the invention, proteins contained in the resulting amide compound-containing solution are analyzed. The protein concentration is determined by using a protein analysis kit produced by Biorad Laboratories, Inc. after the amide compound contained in the amide compound-containing solution is removed by dialysis using a semipermeable membrane, whereby the removal rate of proteins.

Example A

100 ml of a culture medium of the following composition was prepared in a 500-ml Erlenmeyer flask with a baffle and was sterilized in an autoclave at 121°C for 20 minutes. After adding ampicillin to the culture medium to make a final concentration of 50 μg/ml, one platinum loop of the fungus body of clone No. 3 obtained by Example 3 of the specification was planted and cultured at 37°C and 130 rpm for 20 hours. Only the fungus body was separated from the culture liquid by centrifugal separation (15,000 G for 15 minutes), and after again suspending the fungus body in 50 ml of physiological saline, a wet fungus body was obtained by again conducting centrifugal separation.

Culture Medium Composition

Yeast extract	5.0 g/L

Polypepton	10.0 g/L
NaCl	5.0 g/L
Cobalt chloride hexahydrate	10.0 mg/L
Ferric sulfate heptahydrate	40.0 mg/L
pH 7.5	

1.5 g of the wet fungus body obtained in the foregoing was suspended in 98.5 g of 0.3 mM-NaOH aqueous solution, and 60 g of acrylonitrile was added to the suspension at once, followed by carrying out the reaction under agitation at 10°C. After 24 hours from the start of the reaction, the reaction solution was analyzed by the HPLC analysis. As a result, only acrylamide (concentration: 50% by weight) was present in the reaction solution but no acrylonitrile was confirmed. The pH of the hydration reaction solution was 8.0.

The reaction solution was adjusted to pH 4 by a 10% sulfuric acid aqueous solution, to which 2% by weight based on reaction solution of activated carbon (powdery activated carbon PM-SX produced by Mitsukura Chemical Co., Ltd.) was added, and after agitating at 25°C for 5 hours, filtration was carried out by using filter paper.

The protein concentration of resulting filtrate was measured, whereby the removing rate of protein was calculated.

The protein removing rate in amide solution after purification was illustrated in following Table.

Example B and C

The same procedures as in Example A were carried out except that the hydration reaction solution obtained in Example A was adjusted to pH described in the following Table by a 10% sulfuric acid aqueous solution, so as to obtain a filtrate. The protein

removing rate in amide solution after purification was summarized in following Table, with those of Example 3 and 4 and Comparative Example 1 of the specification in the application.

Table

	Before purification	Example 4	Example A	Example B	Example 3	Example C	Comparative Example 1
pH	8	3	4	4.5	5	6	7
Removing rate (%)		76	87	98	99	99	25

Result

From the above Table, it can be understood that, as pH of the amide solution goes up from 3 to 4, the removing ratio of protein after purification become high, and as pH goes up from 6 to 7, it suddenly becomes low.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: March 17, 2009

Kiyoshi Ito